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### Thermal Properties of Mineralized and Non Mineralized Type I Collagen in Bone

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#### ABSTRACT

The research about the structural stability of bone, as a composite material, compromises a complete understanding of the interaction between the mineral and organic phases. The thermal stability of human bone and type I collagen extracted from human bone by different methods was studied in order to understand the interactions between the mineral and organic phases when is affected by a degradation/combustion process. The experimental techniques employed were calorimetry and infrared spectroscopy (FTIR) techniques. The extracted type I collagens result to have a bigger thermal stability with a Tmax at 500 and 530 Celsius degrees compared with the collagen present in bone with Tmax at 350 Celsius degrees. The enthalpy value for the complete degradation/combustion process were similar for all the samples, being  $8.4 \pm 0.11$  kJ/g for recent bones diminishing with the antiquity, while for extracted collagens were  $8.9 \pm 0.07$  and  $7.9 \pm 1.01$  kJ/g. These findings demonstrate that the stability loss of type I collagen is due to its interactions with the mineral phase, namely carbonate hydroxyapatite. This cause a change in the molecular properties of the collagen during mineralization, specifically in its cross-links and other chemical interactions, which have a global effect over the fibers elasticity, but gaining tensile strength in bone as a whole tissue. We are applying this characterization to analyze the diagenetic process of bones with archaeological interest in order to identify how the environmental factors affect the molecular structure of type I collagen. In bone samples that proceed from an specific region with the same environmental conditions, the enthalpy value per unit mass was found to diminish exponentially with respect to the bone antiquity.

#### INTRODUCTION

Bone is one of the biological structures that has been analyzed in different areas, as medicine, biology, archaeology, science materials, etc. by means of different techniques. It is composed of a mineral and an organic phase, which are hydroxyapatite and collagen respectively. This biomaterial properties are of main importance for developing new materials that mimics its structure and for other applications in which it plays a specific and transcending role. To understand its structural characteristics, as the collagen-hydroxyapatite relationship, the collagen thermal stability, the collagen degradation process, we have, in a recent work, address these problems by the use of different calorimetric, gas chromatography and FTIR techniques (6). These findings demonstrate that the stability loss of type I collagen is due to its interactions with

the mineral phase, namely carbonate hydroxyapatite, since its enthalpy value for the complete degradation/combustion process of mineralized collagen and bone extracted collagen are similar,  $8.4 \pm 0.1$  and  $8.9 \pm 0.07$  kJ/g respectively, but their Tmax values differ, being bigger for the extracted collagen. This cause a change in the molecular properties of the collagen during mineralization, specifically in its cross-links and other chemical interactions, possibly provoked by the hydroxyapatite crystals which acts as degradation centers accelerating the protein combustion. The changes in this biomaterial structure properties, have a global effect over the fibers elasticity, but gaining tensile strength in bone as a whole tissue. We pretend to apply these findings to the characterization of the collagen loss process (diagenetic process) in archaeological bone, to develop a calorimetric approach to the dating of bone remains, that can be found in specific environmental conditions.

Type I collagen of archaeological bone has been a matter of several investigations mainly because it is a source of valuable information for understanding the diagenetic process [1,2,4,5,7,8], which is variable from one environment to another, and depends on different physical, chemical and biological factors. It is well known that both, the inorganic and organic phases of bone, are altered during the time this material expends buried. Differential scanning calorimetry has been used for investigating the deterioration of archaeological and actual bone collagen by analysing the denaturation of the extracted protein [7]. Our approach consists in obtaining the  $\Delta H$  of combustion of the collagen molecule present in the bone structure and comparing it with remains of different antiquity, assuming that older bones will have less protein than younger ones because of the degradation provoked by various factors [9]. Studying the combustion process implies the use of a larger temperature range of analysis than with the protein denaturation process, and it also has the outstanding benefit of obtaining satisfactory signals and reproducible results for all the archaeological bones, which has been a difficulty in other studies [7].

Understanding the diagenetic process may provide a better conceptual framework to obtain a possible relationship between the antiquity and the collagen loss in archaeological bones. Due to the fact that the diagenetic process is provoked by multiple factors and that there is no very clear consensus for the particular chemistry involved in any given diagenetic process [4], it turns very difficult to describe it meticulously. Then, the collagen mass of antique bones must be analyzed in a way that allows the obtainment of a realistic collagen loss and bone antiquity relationship. For achieving this, an initial prerequisite is to work with bone samples from the same region to assure that the environmental conditions are nearly equal for all the remains.

## EXPERIMENTAL DETAILS

Archaeological bone samples were supplied from the Anthropological Research Institute at National Autonomous University of Mexico and Autonomous University of Baja California, all were from the Mexico Valley, except Xcambo and Bonampak from Yucatan and Chiapas respectively. The samples were suitable to perform the different experiments. The archaeological bones were powdered in an agate mill and sieved through a 325 mesh. The paleontological bone sample was analyzed using a fragments of 10 mg. DSC measurements were carried out in a Thermal Analysis System 9900, Du Pont 910 (DSC module). For DSC measurements the heating rate was constant and equal to  $10^\circ\text{C}/\text{min}$  in air atmosphere. The sample amount used was 2.0 mg for all archaeological samples. The FT-IR analysis were carried out using a Nicolet 680. The bone samples were mixed with KBr powder (1:100 ratio) and then compressed into pellets. IR spectra were obtained for different samples.

Table 1. Antiquity and enthalpy values of archaeological samples (femur bone).

SAMPLE (origin)	ENTHALPY (J/g)	ANTIQUITY (years)	BONE
Tlatilco Ent. 27	-688.1 ± 27.11	3150 ± 150	Femur
Tlatilco Ent. 197	-1202.27 ± 86.64	3150 ± 150	Femur
Cuiculco A.	-422.06 ± 70.60	2250 ± 350	Femur
Cuiculco I.	-313.33 ± 35.84	2250 ± 350	Femur
Oztoyohualco Ent. 14	-1586.86 ± 27.90	1450 ± 100	Femur
Xcambo	-688.34 ± 31.29	1350 ± 150	unknown
Bonampak	-315.06 ± 37.29	1350 ± 150	unknown
Tlalocan Ent. 2	-471.61 ± 124.91	1300 ± 100	Femur
Xochimilco Ent. 17	-1613.16	1250 ± 150	Femur
Tlatelolco C6 T8	-1431.14 ± 197.70	614.5 ± 135.5	Humerus
Xochimilco Ent. 14-A	-1476.43 ± 93041	614.5 ± 135.5	Femur
San Jerónimo 1	-2224.56 ± 82.51	274.5 ± 99.5	Femur
San Jerónimo 3	-2522.86 ± 14.11	274.5 ± 99.5	Femur
San Pedro San Pablo U1 Ent. 1	-2153.66 ± 159.91	185 ± 25	Femur
San Pedro San Pablo U7 Ent. 5	-814.96 ± 90.03	185 ± 25	Femur
San Pedro San Pablo U7 Ent. 16	-1860.98 ± 115.64	185 ± 25	Femur
San Pedro San Pablo U7 Ent. 18	-1596.79 ± 27.14	185 ± 25	Femur

## DISCUSSION

The calorimetric curves of the archaeological bone samples (fig 1, left graphic; table 1), show that all of them have a similar thermal stability, defined by the Tmax between 330 and 345°C of the combustion process, which represents the temperature where the maximum value of heat flow intensity the process reach. In a previous work we found that the right shoulder in the calorimetric curve of the actual bones, changes if the sample experimental conditions are varied,

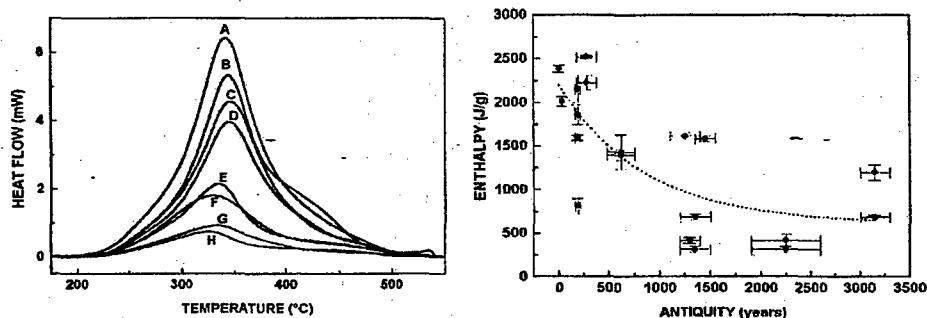


Figure 1. Left graphic shows the archaeological bone samples calorimetric curves. The different curves represent samples with distinct antiquities (A=274, B=185, C=1450, D=614, E=3150, F=1300, G=2250 and H=1350, all in years). Right graphic shows the relationship between the enthalpy and the antiquity of all the bone samples studied, the exponential decay curves shows the general tendency for the collagen loss.

i.e. the use of small bone fragments rather than powdered bone; this is explained by the fact that the DSC analysis is very sensible to the sample conditions, taking this in account all samples were analyzed with the same conditions. The fact that the archaeological bones calorimetric curves gradually diminish is because the thermograms heat flow intensity variation depends on the collagen mass of the bone remains, and the overall process is then represented by smaller calorimetric curves when the collagen loss of the structure is bigger.

The DSC curves shape is not very useful for a good comparison between the samples and their antiquity. This is best represented by the enthalpy values obtained for each bone. When these values varies in modern bones, the organic/mineral phase ratio may be altered, and so the structure properties. In this study, we employ a different approach for interpreting these values, which, as shown in table 1, are not in concordance with the respective antiquity of the bones under study, since we expected to found that the older samples will have lower enthalpy values and vice versa.

In fig. 1 (right graphic) the enthalpy values for all the bone samples are placed against their antiquity and represents the global behavior of the analyzed bones, it also shows that a larger group of samples is needed to fulfill the complete range of antiquities. The enthalpy values for all the bone samples are placed against their antiquity, and an exponential decay curve is obtained which represents the collagen loss rate. The archaeological bones came from the same region to assure that the environmental conditions that act over the collagen molecules degradation is similar, this is not well represented, since some sample of the same antiquity have different enthalpy values, so that the protein degradation process carry out by the soil characteristics (pH, humidity, temperature, etc.) may not be equal for all the bone remains. It is expected that some bone samples may not have the same collagen loss, but a general tendency of such loss rate must be represented by the exponential decay curve in the range of antiquity analyzed.

FTIR analysis also shows that archaeological bones have different collagen loss (fig.2). By comparing the amide I band ( $1650\text{ cm}^{-1}$ ), which is assigned to the C=O stretch, is observed that there are different organic content for the samples. A collagen and a hydroxyapatite spectra are compared with the archaeological bones, the mineralization of the later samples differs

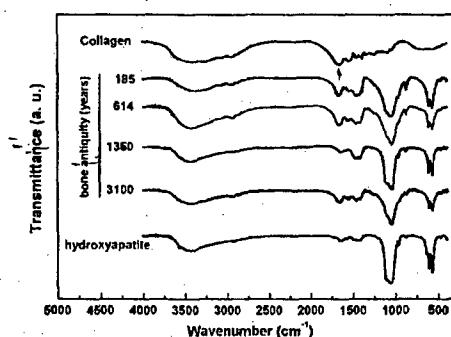


Figure 2. FTIR spectra of bone extracted collagen, hydroxyapatite and archaeological bone samples of different antiquity. The arrow shows the amide I band at  $1650\text{ cm}^{-1}$  that is assigned to the C=O stretching.

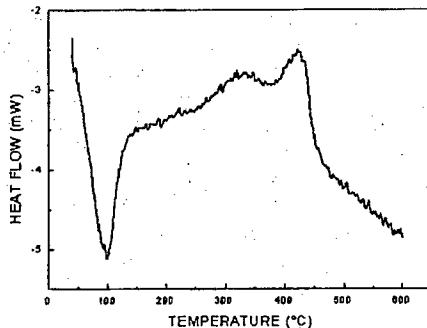


Figure 3. Calorimetric curve of a 73 millions old paleontological bone. Two exothermic processes are evident at 330 and 420 °C.

with their respective antiquity, this may be a consequence of the burial conditions in which the remains were found. The 1350 years old sample (Bonampak) is very similar to the hydroxyapatite spectra, suggesting that the degree of collagen loss is bigger and that the mineral phase does not suffer any kind of modification.

We found that the calorimetric analysis have a greater sensibility for detecting the organic presence in the bone material. To illustrate the sensitivity of the DSC module, let just notice that it was able to detect the presence of the organic phase in some paleontological bones of even 73 million years old (fig.3). This fact confirms that the calorimetry approach is suitable for fossil samples.

The present results may be considered as a first approach to the study of bone changes by the combustion process of the organic phase. It must be taken in consideration that collagen is the principal constituent of bone, so by analyzing the organic phase we are primarily characterizing this specific protein which by the way, is expected to be the unique organic remain in the older bones structure, when external contamination is negligible, because of his great stability. The improvement of the present research may be achieved by the use of a bigger set of bone samples with different antiquities, so that the exponential decay curves may represent in a more precise way the collagen loss rate. It is also very important to characterize this collagen loss rate in different burial conditions that may provoke an accelerated degradation process and loss of the organic content.

#### CONCLUSION

We have applied our recent findings on the thermal stability and degradation process of collagen, and its relationship with the hydroxyapatite phase in the bone structure, to the analysis of archaeological bone. By the use of DSC analysis, we obtain the collagen loss rate from the enthalpy values of the combustion process of the organic phase present in bones. The enthalpy of the combustion process from the archaeological samples has in some cases, no direct concordance with their respective antiquities. By studying a bigger set of bone sample of different antiquities, this calorimetric approach could be employed, as an alternative technique, for the analysis of bone remains of archaeological and paleontological interest.

Material science research may have different applications in areas where structural knowledge and the relationship between different phases in a material exist, and can give useful information to develop a more robust conceptual framework about specific processes. Paleontology and archaeology, are two scientific areas where the characterization of the properties of an important biomaterial are of trascendence to achieve a better understanding of the changes produced in bone by external factors.

Calorimetry is, as shown by the present results, a very useful technique for biomaterials study. The organic content in bone tissues of millions years old, can be detected and correlated to their antiquity, these must be carefully interpreted with the burial context where the remains are found. The use of calorimetry with other commonly used techniques may help in this aspect.

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